Urinalysis: Current Status and Prospects for the Future*

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ABSTRACT

More than 300 million routine clinical analyses are performed annually in the United States. Methods for routine clinical urine examination, including detection of bacteriuria, are briefly reviewed. Prospects of some newer, better techniques to carry out such analyses are introduced. A preliminary report is presented on the use of supravital microscopic fluorescence technique (SMFT), employing acridine orange as a non-specific staining fluorochrome. Results of examining 218 unspun urine specimens by SMFT are compared to traditional bacteriologic culture at a large pediatric hospital reference laboratory.

Introduction

Analysis of urine has been considered an essential part of the examination of a patient since 4000 B.C.† Each year, more than 300 million routine clinical urinalyses are performed in the United States. Urinalysis in its broadest sense includes the physical, chemical or microscopic inspection of urine. This paper addresses primarily routine clinical urine examination, including the detection of bacteriuria, and presents prospects of some newer techniques to perform such analyses more effectively. The biochemical inspection of urinary constituents for detection of disordered metabolism, eg. aminoacidopathies, mucopolysaccharidoses, etc. or the testing for drugs and drug metabolites, is not within the focus of this article.

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Routine Urinalysis

Routine urinalysis, as in all laboratory testing on humans, proceeds through the process of subject selection, ordering, specimen procurement, specimen transport, preparation, analysis and reporting. Fashions in urine testing have changed considerably since 1679, when Thomas Willis described the taste of diabetic urine as “wonderfully sweet as if it were imbued with honey or sugar.” Many older physicians and clinical scientists have lived through the era of gently boiling urine in a test tube over a Bunsen burner, or they have used Fehling’s solution or Benedict’s solution to test for reducing sugars in urine.

In the early 1940s, a major advance in the laboratory analysis of urine occurred when commercially-prepared copper reduction tablets were first used in the laboratory. Copper sulfate, sodium hydroxide, citric acid and carbonate were pressed as a tablet. The tablet provided its own heat for the Benedict’s cupric ion to cuprous ion chemical reaction with urine. Reducing sugars would change the color from blue to orange-red, depending upon the amount of reducing substance in the urine.

The introduction of urine chemistry reagent strips with complex chemical reactions incorporated into a paper “solid phase” matrix dramatically and forever changed urine testing and screening to a simple dip and “read” process. At first, in 1956, only glucose was measured by this dipstick technique. Protein and ketone testing was added to a single strip in 1957. Later, pH (1959), blood (1961), bilirubin and urobilinogen (1969) measurement and specific gravity assessment (1981) were developed as dipstick tests.

In 1972, nitrite was added to the spectrum of urine dipstick tests. A positive nitrite test indicates that bacteria, which are capable of reducing nitrate in urine, are present in “significant” numbers. Unfortunately, not all bacteria convert nitrate to nitrite.

Introduction of the leukocyte esterase dipstick to the paper dipstick array of screening tests in 1984 provided a means of estimating the white blood cell content of urine. Unfortunately, neither the nitrite nor the leukocyte esterase dipstick test is considered as reliable an indicator of urinary tract infection as detection of bacteriuria by traditional Gram’s stain or the use of the extant “gold standard” of bacterial detection via bacterial culture of a catheterized urine specimen or a fresh midstream “clean catch” specimen. In practice, the ease and simplicity of dipstick testing frequently outweigh the complexity of and time required for “gold standard” evaluation.

Routine urinalysis can be performed in vastly different settings: by large commercial reference laboratories, handling thousands of tests daily, by smaller commercial urine laboratories, by hospital clinical laboratories or by physicians’ office laboratories. A laboratory technician, physician or nurse can perform such testing.

For detection of bacteriuria, specimen collection ideally requires a midstream urine specimen in a clean container. Cleansing of the genital area prior to collection is advised. Quantitative loop inoculation of a well-shaken urine sample to bacterial culture medium soon after collection is desirable. If storage of the specimen is required, collection in a closed sterile container, preferably with storage time not exceeding a few hours and temperatures maintained at 4°C, has been recommended.

For microscopic examination of urine, 10, 12, or 15 ml of properly collected urine is centrifuged at 450 g for five minutes. The supernatant is removed and preserved for use in diluting the sediment sample if microscopic examination is required. Microscopy of the sediment can be performed by bright-field, phase contrast or polarized light visual analysis.

The diagnosis of urinary tract infection is based on a quantitative estimation of the concentration of bacteria in the urine specimen. An assumption that each bacterium multiplies to become a colony has led to calling these “colony-forming units,” (CFU). In an asymptomatic individual, more than 100,000 CFU/ml in an adequately collected specimen usually indicates urinary tract infection; in
patients with symptoms, the threshold can be as low as 1000 CFU/ml. Although uninfected bladder urine is sterile, samples collected by the spontaneous passage of urine are never sterile because the cleansing process of the genital area is not completely efficient. Therefore, bacterial colonies generally will be found in the urine of uninfected, as well as infected, patients.6

Automation

Urine chemistry dip-and-read tests are technically chemically complex, but the process itself is certainly convenient and easy and can be performed by anyone with normal color vision. Since 1956, well over 20 billion dipsticks have been manufactured. They are used extensively in a variety of situations, eg, in hospitals, clinics, physicians' offices and homes of patients, and by individuals with diverse levels of education and training.

Several types of automated and semiautomated urine analyzer instruments have been developed to "read" chemical tests on dipsticks by reflectance spectroscopy, to standardize readings and, very importantly, via computer interface, to store data and print out results. Since 1997, such reagent strip urine analyzers have been advanced to measure human chorionic gonadotropin (hCG) levels to serve as a fast, reliable way to test for pregnancy. Microalbumin reagent strips have also been developed to identify patients with early stages of kidney damage. The hCG and microalbumin reagent strips, however, require the use of a semiautomatic dedicated instrument analyzer.

Many automated and semiautomated urine analyzers are currently available. The majority were developed in the mid-1980s and are used mainly in large hospital and commercial laboratories. These analyzers can cost more than $100,000. Their specimen capacity (throughput/hour) can vary from 25 to greater than 250. Microscopic analysis is not available in most urine analyzers; slideless microscopy is available in a few. Slideless microscopy provides a profile of cells and microorganisms present in a urine specimen. However, if the profile indicates an abnormally high level of microorganisms, routine brightfield urine microscopy is still recommended.

Prospects For the Future of Automated Urine Analyzers

At a meeting of the American Society for Microbiology in 1998, a fully automated computer-assisted, optically-based, in vitro diagnostic instrument intended for rapid diagnoses of bacteria in urine was reported and is currently still under development. Optical properties of single cells are used for quantitative measurement by computerized image analysis techniques. Apparently, this instrument can provide antibiotic susceptibility testing in a matter of two to three hours and is capable of performing bacterial screening and microorganism identification via monoclonal antibodies. This may well be the prototype instrument for use in large-scale reference testing laboratories. This system has the potential of automating completely the evaluation of urine specimens for the presence of bacteria. Such a process would be time- and labor-saving and, very importantly, would eliminate the need for culturing negative specimens.

Data Interpretation

In the 1950s, Kass conducted studies which resulted in the concept that a urinary tract infection is likely to be present if bacterial culture of a clean voided specimen grows a single bacterial species with a colony count ≥100,000 colony-forming units (CFU) per milliliter. His contributions, along with those of others, led to the universal adoption of quantitative cultures for the diagnosis of a urinary tract infection.7-9 Varying criteria for "significant" bacteriuria are indicated for catheterized and "clean catch" specimens.

There is a tendency now to move urine testing away from the physician's office laboratory. With the transport of specimens to a commercial laboratory, an era of urine practice has evolved where broad-spectrum antimicrobials
are immediately prescribed while awaiting results of urine culture and sensitivity testing 24 to 48 hours later. The time lapse from the initial patient examination and receipt of test results frequently blunts the physician’s interest and, consequently, the attention given the laboratory analysis. More importantly, it is generally known that 30 percent of urine samples submitted for culture are reported to have a “significant” urine colony count of \( \geq 100,000 \text{ CFU/ml} \). When more than one species of microorganism is detected, even these elevated counts may be interpreted as being contaminants, rather than specific etiologic agents of urinary tract infection.

To accept such “quantitative” colony counts “blindly” does not necessarily represent valid data interpretation. To ignore the specificity and virulence of the infecting microorganisms involved in a urinary tract infection invites therapeutic failure. Neither should the suggestion that culture of the urinary sediment requires, “prior” washing of the sediment to dilute inhibitory factors that may be present in raw urine be lightly dismissed.\(^\text{10}\)

Microscopic Examination of Urine

The microscopic examination of urine has not changed appreciably for over half a century. Centrifuged sediment from 10 to 15 ml. of urine is examined by brightfield microscopy, using a simple glass slide and glass cover-slip preparation. Detection of an excessive number of microorganisms and/or excessive numbers of white blood cells (WBCs), coupled with information derived from dipstick analysis, frequently forms the basis in the decision to culture or not to culture a specific specimen.

Enhanced Urine Microscopic Examination

In 1982, Corman et al, advocated the examination of fresh, unspun, unstained urine for bacteria on a counting chamber. Using a Neubauer hemocytometer counting chamber and a high dry objective (450x), they quantitatively counted WBCs, rods and cocci in chains (they were unable to differentiate a single coccus from amorphous crystals). They advocated the use of this method as highly accurate for detecting bacteriuria. Currently several laboratories, particularly in Europe, employ modifications of this procedure for counting WBCs and bacteria.\(^\text{11}\)

Supravital Microscopic Fluorescence Technique (SMFT)

For over a decade, the use of the supravital microscopic fluorescence technique (SMFT) has been advocated for the screening detection of microorganisms (bacteria, including mycoplasma, spirochetes, fungi, parasites, etc.) in a wide spectrum of clinical applications.\(^\text{12-14}\) This technique requires less than two minutes for sample preparation since it is basically a simple wet-slide preparation involving the addition of equal amounts of unspun urine and a fluorochrome (eg, acridine orange in a sterile phosphate buffer solution). The edges of the glass cover-slip preparation are then sealed with melted paraffin. Using an epi-fluorescence microscope, a 40x and/or a 100x oil objective, it is ready for immediate visual inspection and interpretation.

In 1995, Lorincz, Baltaro and Adamson reported detection of significant bacteriuria using SMFT. Uncentrifuged urine samples from 77 patients ranging in age from 54 to 99 years were examined by SMFT and compared to standard urine cultures. Of the 77 specimens, 71 (92 percent) were accurately identified as to whether “significant” bacteriuria was present. Only one of the 17 specimens that had significant bacteriuria was not detected by SMFT examination.\(^\text{15}\)

SMFT Urine Examination in Children

The authors are presently engaged in a considerably larger prospective study to compare the SMFT screening of uncentrifuged urine to traditional reference laboratory urinalysis and bacterial culture. The preliminary results of 218 samples are reported here. All SMFT evaluations were made by two investigators:
AEL, an experienced fluorescence microscopist, and GCD, a premedical student with minimal brightfield microscopy experience and no prior familiarity with an epifluorescence microscope. The SMFT slide preparation and examination were executed individually, and the elapsed time from slide preparation to reporting of SMFT results rarely exceeded five to seven minutes. A simple decision was rendered for each SMFT evaluation, namely, whether or not urine culture was indicated. Criteria were semiquantitative, and if microorganisms were present in greater than 1/hpf and/or the number of white blood cells were greater than 1/hpf, then culture was considered.

All SMFT evaluations were conducted on refrigerated, well-shaken, unspun urine samples within 48 hours of collection by the hospital laboratory. The SMFT examiners had no medical record information, nor did they have data with respect to indications for urine testing, use of antimicrobials, results of dipstick analyses or other laboratory tests. Interobserver error for the two SMFT evaluators was insignificant.

For the purpose of this preliminary data analysis, cultures performed at the hospital reference laboratory that had greater than 100,000 CFU/ml, including a combined colony count of potential pathogens, were considered positive. Cultures with counts less than 100,000 CFU/ml were considered negative.

Results of the first samples evaluated follows:

<table>
<thead>
<tr>
<th>Hospital Culture</th>
<th>SMFT Evaluation</th>
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<tbody>
<tr>
<td>greater than 100,000 CFU/ml</td>
<td>Culture Indicated</td>
</tr>
<tr>
<td>less than 100,000 CFU/ml</td>
<td>38 (17.4%)</td>
</tr>
<tr>
<td></td>
<td>14 (6.4%)</td>
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</tbody>
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Predictive value of a positive test = 73%
Predictive value of a negative test = 93%
Sensitivity = 83%
Specificity = 92%

McNemar's test comparing SMFT evaluation to bacterial culture as paired samples failed to reject the hypothesis that these two methods differed from each other. P value = 0.28.

Figure 1b demonstrates the overwhelming capability of vitally stained material, as viewed by epifluorescence, to reveal exquisite detail of cellular and nuclear morphology, as well as discrete individual microorganisms. Since the organisms and cells are alive, their mobility patterns may also be observed. Moreover, microorganisms less than 1 μ in diameter, e.g., mycoplasma that are beyond the resolution of a brightfield microscope, can be readily examined by epifluorescence. The same field is viewed by brightfield illumination in figure 1b, and here even detection of the large epithelial cell becomes a major challenge. Since a high dry 40x objective is currently used for most urine microscopy, the severe limitations of such brightfield microscopic examinations are even more evident.

Prospects For the Future of SMFT Urinalysis

SMFT screening of urine has the potential for automation for large-scale use. Even though sample preparation for the supravital microscopic technique is now very simple, the day may soon come when self-staining, readily disposable, non-glass slides are available for epifluorescence study.

Additional Advantages of Supravital Microscopic Technique

SMFT is very inexpensive, has a rapid turnaround time, can be performed at point of care and is relatively user-friendly for anyone with minimal microscope skills. The screening technique is scientifically more accurate. The test is reproducible and requires less than 100 μl of specimen. The procedure is easily taught and easily learned. It can accurately eliminate unnecessary urine cultures in 70 percent or more cases, which further enhances its cost-
effectiveness. The ability to view vitally stained microorganisms can achieve much of the same results as Gram’s stain preparations. Unfortunately, as we have reported here, SMFT nonspecifically stains all cells and does not detail the potential susceptibility of the microorganisms detected to microbials. As documented, there is a possibility that SMFT may be substituted for darkfield microscopic examination since spirochetes can be readily visualized. Similarly, yeasts and fungi can be easily identified, so that the traditional KOH preparation could be replaced. Also significant is the ability of SMFT to determine nuclear morphology of living cells.

Today, when we are attempting to solve managed care problems with yesterday’s technology, the prospect of better-developed techniques to carry out meaningful laboratory testing looms fluorescently brighter than ever for routine urinalysis.

Ultimately, as in all laboratory analysis, “Die methode ist alles” (the method is everything). Nor should we forget that the real reason for
urine laboratory testing should be to improve the outcome for the patient's health. Now is the time to establish a solid research agenda to measure and monitor these important outcomes and, most critically, to change practices when results so indicate.2

References